

## CLAIMS

1. A method of measuring an amount of a glycated protein in a sample,  
comprising:
  - 5 causing a fructosyl amino acid oxidase to act on a glycated amino acid present in the sample other than the glycated protein so as to remove the glycated amino acid by degrading it; then
  - causing a fructosyl amino acid oxidase to act on the glycated protein to cause a redox reaction in the presence of a tetrazolium compound and
  - 10 sodium azide; and
  - measuring the redox reaction to determine the amount of the glycated protein.
2. The method according to claim 1, wherein the glycated protein is glycated  
15 hemoglobin.
3. The method according to claim 1, further comprising:
  - degrading the glycated protein with a protease to give a degradation product of the glycated protein either before or after causing the fructosyl
  - 20 amino acid oxidase to act on the glycated amino acid,
  - wherein the fructosyl amino acid oxidase caused to act on the glycated protein is caused to act on the degradation product to cause the redox reaction.
- 25 4. The method according to claim 1, wherein the measurement of the redox reaction is measurement of an amount of hydrogen peroxide formed by causing the fructosyl amino acid oxidase to act on the glycated protein, the measurement of the amount of the hydrogen peroxide comprising:
  - adding N-(carboxymethylaminocarbonyl)-4,4'-bis(dimethylamino)
  - 30 diphenylamine sodium salt as a color-developing substrate to a reaction solution of the redox reaction in the presence of a surfactant, thereby causing a redox reaction between the color-developing substrate and the hydrogen peroxide; and
  - measuring an amount of color developed by the color-developing
  - 35 substrate to determine the amount of the hydrogen peroxide,
  - wherein, a concentration of the tetrazolium compound in the reaction solution is in a range from 0.5 to 8 mmol/l, a concentration of the sodium

azide in the reaction solution is in a range from 0.08 to 0.8 mmol/l, a concentration of the surfactant in the reaction solution is in a range from 0.3 to 10 mmol/l, and a pH of the reaction solution is in a range from 7.0 to 8.5.

- 5 5. The method according to claim 1, wherein the fructosyl amino acid oxidase caused to act on the glycated amino acid is specific for a glycated  $\alpha$ -amino group, and the fructosyl amino acid oxidase caused to act on the glycated protein is specific for a glycated  $\alpha$ -amino group and a glycated side chain of an amino acid residue.
- 10 6. The method according to claim 1, wherein a solution containing the tetrazolium compound and the sodium azide is aged and is then added to the sample.
- 15 7. The method according to claim 1, wherein the tetrazolium compound is 2-(4-iodophenyl)-3-(2,4-dinitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium salt.
- 20 8. A method of determining a ratio of glycated hemoglobin to hemoglobin, comprising:  
measuring an amount of glycated hemoglobin in a sample by the method according to claim 1;  
measuring an amount of hemoglobin in the sample; and  
calculating the ratio of the glycated hemoglobin to the hemoglobin  
25 using the amount of the glycated hemoglobin and the amount of the hemoglobin thus measured.
9. A measuring kit used for measuring a glycated protein using a redox reaction, comprising:  
30 a pretreatment reagent for pretreating a sample, containing a fructosyl amino acid oxidase; and  
a color-developing reagent containing a fructosyl amino acid oxidase, an oxidoreductase, and a color-developing substrate.
- 35 10. The measuring kit according to claim 9, wherein the glycated protein is glycated hemoglobin.

11. The measuring kit according to claim 9, wherein the fructosyl amino acid oxidase contained in the pretreatment reagent is specific for a glycated  $\alpha$ -amino group, and the fructosyl amino acid oxidase contained in the color-developing reagent is specific for a glycated  $\alpha$ -amino group and a glycated side chain of an amino acid residue.
12. The measuring kit according to claim 9, further comprising a protease reagent containing a protease.
13. The measuring kit according to claim 12, wherein the protease is at least one protease selected from the group consisting of metalloproteinases, bromelain, papain, trypsin, proteinase K, subtilisin, and aminopeptidase.
14. The measuring kit according to claim 12, wherein the protease is at least one protease that degrades glycated hemoglobin selectively and is selected from the group consisting of metalloproteinases, bromelain, papain, trypsin derived from porcine pancreas, and protease derived from *Bacillus subtilis*.
15. The measuring kit according to claim 12, wherein the protease reagent further contains a tetrazolium compound and sodium azide.
16. The measuring kit according to claim 15, wherein, in the protease reagent, the tetrazolium compound (A) and the sodium azide (B) are present at a ratio (molar ratio A : B) in a range from 20 : 3 to 20 : 12.
17. The measuring kit according to claim 12, wherein the protease reagent contains a metalloproteinase as the protease and further contains Ca and Na, and a concentration of the metalloproteinase is in a range from 100 to 40,000 U/l, a concentration of Ca is in a range from 0.1 to 50 mmol/l, and a concentration of Na is in a range from 5 to 1000 mmol/l.
18. The measuring kit according to claim 9, wherein the color-developing substrate is N-(carboxymethylaminocarbonyl)-4,4'-bis(dimethylamino) diphenylamine sodium salt.
19. The measuring kit according to claim 9, wherein at least one of the

pretreatment reagent and the color-developing reagent further contains a surfactant.

20. The measuring kit according to claim 12, wherein the protease reagent  
5 further contains a surfactant.

21. The measuring kit according to claim 19 or 20, wherein the surfactant is  
at least one surfactant selected from the group consisting of polyoxyethylene  
ethers, polyoxyethylene phenol ethers, polyoxyethylene sorbitan alkyl esters,  
10 and polyoxyethylene alkyl ethers.

22. The measuring kit according to claim 9, wherein the pretreatment  
reagent further contains at least one buffer selected from the group consisting  
of CHES, MOPS, TAPS, EPPS, phosphate, HEPPSO, POPSO, and borate,  
15 and a pH of the pretreatment reagent is in a range from 8.0 to 10.0.

23. The measuring kit according to claim 9, wherein the color-developing  
reagent further contains at least one buffer selected from the group consisting  
of MES, Tris, phosphate, MOPS, TES, HEPES, HEPPSO, and EPPS, and a  
20 pH of the color-developing reagent in a range from 6.0 to 9.0.

24. The measuring kit according to claim 12, wherein the protease reagent  
further contains at least one buffer selected from the group consisting of Tris,  
MES, DIPSO, TES, POPSO, HEPES, MOPSO, Bis-Tris, MOPS, ADA, PIPES,  
25 ACES, and phosphate, and a pH of the protease reagent is in a range from 5.0  
to 7.0.

25. The measuring kit according to claim 15, wherein the tetrazolium  
compound is 2-(4-iodophenyl)-3-(2,4-dinitrophenyl)-5-(2,4-disulfophenyl)-  
30 2H-tetrazolium salt.

26. The measuring kit according to claim 9, wherein the pretreatment  
reagent further contains at least one of uricase and bilirubin oxidase.

35 27. The measuring kit according to claim 9, wherein the color-developing  
reagent further contains sodium azide.

28. The measuring kit according to claim 22, wherein  
the fructosyl amino acid oxidase in the pretreatment reagent is  
specific for a glycated  $\alpha$ -amino group;  
in the pretreatment reagent, a concentration of the fructosyl amino  
acid oxidase is in a range from 10 to 5000 U/l and a concentration of the  
buffer is in a range from 5 to 200 mol/l; and  
a pH of the pretreatment reagent is in a range from 8.0 to 10.0.
29. The measuring kit according to claim 15, wherein  
the protease reagent further contains Ca, Na, and a buffer;  
the protease in the protease reagent is a metalloproteinase;  
in the protease reagent, a concentration of the metalloproteinase is in a  
range from 100 to 10,000 KU/l, a concentration of the tetrazolium compound  
is in a range from 0.1 to 10 mmol/l, a concentration of the sodium azide is in a  
range from 0.08 to 4 mmol/l, a concentration of Ca is in a range from 0.1 to 50  
mmol/l, a concentration of Na is in a range from 5 to 1000 mmol/l, and a  
concentration of the buffer is in a range from 0.1 to 500 mol/l; and  
a pH of the protease reagent is in a range from 5.0 to 7.0.
30. The measuring kit according to claim 23, wherein,  
in the color-developing reagent, the fructosyl amino acid oxidase is  
specific for a glycated  $\alpha$ -amino group and a glycated side chain of an amino  
acid residue, the oxidoreductase is a peroxidase, and the color-developing  
substrate is N-(carboxymethylaminocarbonyl)-4,4'-bis(dimethylamino)  
diphenylamine sodium salt;  
in the color-developing reagent, a concentration of the fructosyl amino  
acid oxidase is in a range from 100 to 50,000 U/l, a concentration of the  
peroxidase is in a range from 0.1 to 400 KU/l, a concentration of the  
N-(carboxymethylaminocarbonyl)-4,4'-bis(dimethylamino)diphenylamine  
sodium salt is in a range from 0.02 to 2 mmol/l, and a concentration of the  
buffer is in a range from 10 to 500 mol/l; and  
a pH of the color-developing reagent is in a range from 6 to 9.